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The effect of topical adrenergic and anticholinergic agents on the choroidal thickness of young healthy adults

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ABSTRACT

The human choroid is capable of rapidly changing its thickness in response to a variety of stimuli. However little is known about the role of the autonomic nervous system in the regulation of the thickness of the choroid. Therefore, we investigated the effect of topical parasympatholytic and sympathomimetic agents upon the choroidal thickness and ocular biometrics of young healthy adult subjects. Fourteen subjects (mean age 27.9 ± 4 years) participated in this randomized, single-masked, placebo-controlled study. Each subject had measurements of choroidal thickness (ChT) and ocular biometrics of their right eye taken before, and then 30 and 60 min following the administration of topical pharmacological agents. Three different drugs: 2% homatropine hydrobromide, 2.5% phenylephrine hydrochloride and a placebo (0.3% hydroxypropyl methylcellulose) were tested in all subjects; each on different days (at the same time of the day) in randomized order. Participants were masked to the pharmacological agent being used at each testing session. The instillation of 2% homatropine resulted in a small but significant increase in subfoveal ChT at 30 and 60 min after drug instillation (mean change 7 ± 3 μm and 14 ± 2 μm respectively; both $p < 0.0001$). The parafoveal choroid also exhibited a similar magnitude, significant increase in thickness with time after 2% homatropine ($p < 0.001$), with a mean change of 7 ± 0.3 μm and 13 ± 1 μm (in the region located 0.5 mm from the fovea center), 6 ± 1 μm and 12.5 ± 1 μm (1 mm from the fovea center) and 6 ± 2 μm and 12 ± 2 μm (1.5 mm from the fovea center) after 30 and 60 min respectively. Axial length decreased significantly 60 min after homatropine ($p < 0.01$). There were also significant changes in lens thickness (LT) and anterior chamber depth (ACD) ($p < 0.05$) associated with homatropine instillation. No significant changes in choroidal thickness, or ocular biometrics were found after 2.5% phenylephrine or placebo at any examination points ($p > 0.05$). In human subjects, significant increases in subfoveal and parafoveal choroidal thickness occurred after administration of 2% homatropine and this implies an involvement of the parasympathetic system in the control of choroidal thickness in humans.

1. Introduction

The choroid is a highly vascularised layer that plays an important role in normal ocular function. It supplies the outer retina with oxygen and nutrients under highly regulated blood flow, acts as a heat diffuser that protects the photoreceptors and also secretes growth factors (Nickla and Wallman, 2010). The choroid also directly modulates the intraocular pressure (IOP) via vasomotor control of blood flow and indirectly through its role in uveoscleral outflow. Given the vascular nature of the tissue, the choroid's morphology is mainly determined by the course and branching pattern of the ciliary arteries (Bill et al., 1983). It also contains non-vascular smooth muscle cells, a network of spindle- or star-shaped cells that are thought to play a role in altering the thickness of the choroid under the influence of local mediators and intrinsic choroidal neurons that receive input from the sympathetic and parasympathetic nervous system (Nickla and Wallman, 2010; Lütjen-Drecoll, 2006).

Advances in spectral domain optical coherence tomography (SD-OCT) technology have enabled the visualisation and reliable measurements of the human choroid (Ikuno et al., 2010; Hirata et al., 2011; Ouyang et al., 2011). Estimates of the average subfoveal choroidal thickness in the healthy eyes of adult populations (age 18 and over) have ranged between 192 and 354 μm dependent upon the age and refractive error distribution of the population

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(Ikuno et al., 2010; Hirata et al., 2011; Ouyang et al., 2011). Some topographical variations in the choroidal thickness across the posterior pole have also been found, with the choroid consistently reported to be thicker superiorly and temporally, compared to nasal and inferior regions (Hirata et al., 2011; Ouyang et al., 2011). Recent clinical studies have found changes in ChT to be associated with a number of ocular diseases, such as central serous chorioretinopathy (Maruko et al., 2011b), polypoidal choroidal vasculopathy (Koizumi et al., 2011), age related macular degeneration (Manjunath et al., 2011), inflammatory eye diseases (Maruko et al., 2011a), inherited retinal dystrophies (Yeoh et al., 2010), diabetic retinopathy (Esmaeelpour et al., 2011), and glaucoma (Maul et al., 2011). Changes in the thickness of the human choroid have also been found associated with age (Manjunath et al., 2011) and the presence of refractive error (Ikuno et al., 2010; Hirata et al., 2011). Short term changes in human choroidal thickness have also been reported, associated with time of the day (Brown et al., 2009; Chakraborty et al., 2011; Tan et al., 2012), smoking (Sızmaz et al., 2013), caffeine intake (Vural et al., 2014) and with imposed retinal image blur (i.e. hyperopic and myopic defocus) (Read et al., 2010; Chakraborty et al., 2012, 2013).

Additionally, evidence from animal studies suggests that short-term changes in the thickness of the choroid associated with retinal image blur are related to longer term changes in eye growth and the development of refractive errors (Wallman et al., 1995). The magnitude of choroidal thickness variations in relation to defocus found in humans (Read et al., 2010; Chakraborty et al., 2012, 2013) and mammals (Hung et al., 2000) are substantially smaller than those found in avian species (Wallman et al., 1995). Furthermore, several non-selective and selective (M4) muscarinic antagonists have been found to cause a transient thickening of the choroid in chicks (Nickla et al., 2013) or to prevent the choroidal thinning that is normally associated with form-deprivation myopia (McBrien et al., 2011). In contrast, a number of non-specific muscarinic agonists stimulated eye elongation and choroidal thinning in intact chicken eyes (Nickla et al., 2013).

Although the exact mechanism underlying reports of short-term changes in choroidal thickness is not known, previous studies have revealed a rich autonomic vasoactive nerve supply to the choroid involving the activation of sympathetic adrenergic and parasympathetic muscarinic receptors (Neuhuber and Schrödl, 2011). Parasympathetic nerves derived from the pterygopalatine ganglion, via the facial nerve, form a dense perivascular plexus around the choroidal vessels and mediate an increase in blood flow by vasodilatation. These fibres also terminate on non-vascular smooth muscle cells and modulate their tonus (Nickla and Wallman, 2010; Neuhuber and Schrödl, 2011). In addition, the choroid receives innervation from the ciliary ganglion, via the oculomotor nerve that terminates on blood vessels and non-vascular smooth muscle. However, the role of these fibres in the regulation of choroidal blood flow in mammals is not well established, compared to birds where they comprise the main parasympathetic input to the choroid (Nickla and Wallman, 2010; Neuhuber and Schrödl, 2011). A dense sympathetic innervation originates from the ipsilateral superior cervical ganglion, its stimulation causes choroidal vasoconstriction mainly via activation of α_1–adrenergic receptors located in the smooth muscle cells of the vessels (Lanigan et al., 1988). Apart from the autonomic innervation, the choroid also receives the sensory fibres that arise from the trigeminal ganglion via ophthalmic nerve that exert a vasodilatatory influence on choroidal blood flow through local effector function-"axon reflex" in addition to the sensory functions (Nickla and Wallman, 2010; Neuhuber and Schrödl, 2011).

The rich autonomic innervation to various choroidal structures suggests a potential involvement of the autonomic system in the regulation of choroidal thickness, either through modulation of choroidal blood flow, and/or alterations in the tone of non-vascular smooth muscle (Nickla and Wallman, 2010).

We have examined the effects of the modification of the autonomic nervous system activity upon choroidal thickness through the use of topical parasympatholytic and sympathomimetic agents. This was measured with SD-OCT at 30 and 60 min after cholinergic blockade or adrenergic stimulation on different days in healthy young adults. By investigating ocular biometry after topical adrenergic and anticholinergic agent administration, we hoped to improve our understanding of the mechanisms regulating the thickness of the choroid in humans.

2. Material and methods

2.1. Subjects

Fourteen young healthy subjects (mean age 27.9 ± 4 years) participated in this randomized, single-masked, placebo-controlled study. The study and protocol conformed to the tenets of the Declaration of Helsinki and was approved by the university human research ethics committee. Subjects were recruited primarily from the students and staff of the university. Sixty four percent of the participants ($n = 9$) were male and 50% were Caucasian (Caucasian $n = 7$, Indian $= 6$, East Asian $n = 1$). Written informed consent was obtained from participants after thorough explanation of the nature and risks of the experiment before commencement of the study. None of the participants had any significant ocular or systemic disease and had no history of ocular injury or surgery.

Before the study, each participant underwent a full eye examination to determine their refractive status and to ensure they met the criteria of good ocular health. All subjects had normal visual acuity of logMAR 0.00 or better. Their mean spherical equivalent refractive error was −0.62 ± 1.42 DS (range −4.00 to +0.75 DS). The majority of participants exhibited low refractive errors (+0.75 to −0.75 DS) with only 2 subjects with myopic refractive errors > −0.75 DS. No participant exhibited anisometropia of greater than 1.00 DS or cylindrical refraction of greater than 1.00 DC. Two of the participants were soft contact lens wearers, but they ceased lens wear one week prior to participation in the experiment and did not wear lenses for the duration of their involvement in the experiment. To limit the potential confounding influence of ocular diurnal variations upon the results (Chakraborty et al., 2011), all three experimental conditions were tested at approximately the same time of day between 9 am and 2 pm on different days, in randomized order, for each subject.

2.1.1. Pharmacologic agents

Only the right eyes were treated with either: one drop of 2% homatropine hydrobromide, or one drop of 2.5% phenylephrine hydrochloride or placebo (consisting of 0.3% hydroxypropyl methylcellulose) over three separate days, with a different pharmacological agent tested at each visit in randomized order. Homatropine hydrobromide is a non-selective anticholinergic agent that is closely related to atropine (although it has shorter lasting mydriatic and cycloplegic effects), while phenylephrine is a selective α_1-adrenergic agonist. The drug doses were chosen based on the dosage that is commonly used in clinical practice, since these doses are known to be safe and to have a clinically significant pharmacological effect on the iris and ciliary body function. The doses are also predicted to exceed the published ED50 values of phenylephrine (Theofilopoulos et al., 1988) and homatropine (Smith, 1976). To prevent contamination of a trial due to the residual action of a previously administered agent, a washout period up to ten times the terminal elimination half-life of the drug was

chosen (World Health Organization, 2004). Both drugs used in the study have terminal half-lives ranging from 2.5 to 3 h and 3.8–4.8 h for phenylephrine and homatropine respectively (Dart, 2004; Gambill et al., 1967). Therefore a washout period of at least 48 h after the use of each agent was implemented. Participants were masked to the pharmacological agent being used at each testing session. All eye drops were instilled into the inferior conjunctival sac, and participants were directed to close their eyes for approximately 1 min following drop instillation to limit loss of medication through the nasolacrimal drainage system and to maximise ocular drug contact.

2.2. Procedures

On each of the three testing days, all subjects had a series of ocular measurements taken before and 30 and 60 min following the instillation of one drop of the pharmaceutical agent. The room lighting was kept at low photopic levels for all subjects throughout the entire protocol (room illuminance was ~10 lux).

The retinal and choroidal thickness measurements were obtained with the SOCT Copernicus HR (Optopol Technology S.A. Zawiecie, Poland). The SOCT Copernicus HR is a spectral domain optical coherence tomographer that provides high-resolution, cross-sectional images of the posterior eye. The instrument uses a super-luminescent diode light source with centre wavelength of 850 nm (bandwidth 100 nm) and has an axial resolution of 3 μm and transverse resolution of 12–18 μm. The Copernicus HR scans the retina and choroid at 52,000 A-scans per second. Recent studies have shown that this instrument provides reliable measurements of both retinal and choroidal thickness (Wolf-Schnurrbush et al., 2009; Benavente-Pérez et al., 2010).

Three series of OCT scans (6 mm length “cross” scans that capture a set of horizontal and vertical line scans each centred on the fovea; with each series of scans consisting of 30 B-Scans, each with 999 A-scans) were collected from the right eye of each participant at each measurement session and were later averaged. To enhance the signal from the choroid, the “chorioretinal” scanning mode of the instrument was used. Only scans with an image quality index (QI) of more than 4 (mean ± SD was 6.3 ± 1.1) were included in analysis to allow reliable detection of the RPE and chorioscleral interface in the analysed OCT images.

Ocular biometrics before and after instillation of each of the three agents were also assessed with a non-contact biometer (Lenstar LS 900; Haag Streit AG, Koeniz, Switzerland). This instrument is based on the principle of low coherence optical reflectometry and it provides accurate and reliable measures of axial length (AL), central corneal thickness (CCT), corneal curvature, lens thickness (LT) and anterior chamber depth (ACD) along the visual axis in a single measurement procedure (Buckhurst et al., 2009; Cruysberg et al., 2010; Holzer et al., 2009). The instrument calculates the axial length based upon the distance between the anterior cornea and the retinal pigment epithelium. The anterior corneal curvature measures are based upon analysis of the image of 32 reference points reflected from the anterior corneal surface oriented in 2 circles at approximately the 2.3 and 1.65 mm optical zones. Since the Lenstar does not allow control of accommodation when viewing the instrument's internal fixation target, the biometric measurements may potentially be influenced by accommodation. Therefore, accommodation control was achieved with a periscope system consisting of a pair of 45° mirrors and trial frame (in order to provide the full distance spherical equivalent refractive correction to the fellow eye) mounted in front of the Lenstar (Fig. 1). The periscope system allowed two different stimuli to be presented to the two eyes (the biometer's internal fixation target to the right eye and an external target at 6 m to the left eye) leading to dichoptic (simultaneous) presentation of the stimuli and therefore allowing the biometric measurements to be captured during relaxed accommodation. Before the measurements were carried out, care was taken to ensure the centre of the external target (a high-contrast, Maltese cross) was aligned precisely with the centre of the instrument's measurement beam to ensure on axis (foveal) measurements.

Thus, the fellow eye viewed a free-space target consisting of a Maltese cross placed at the eye's far point (~0 D accommodative demand) through the trial lens, while the treated (right) eye saw the biometer's fixation target. Subjects were instructed to keep the Maltese cross target in sharp focus throughout all measurements and to report any misalignment between the two targets. Five consecutive measurements were collected from each subject at each measurement session and were later averaged.

IOP was also measured at each session using the Ocular Response Analyser instrument (ORA; Reichert, Depew, NY). The ORA provides reliable IOP measurements: IOPg, which is calibrated against Goldman applanation tonometry and IOPcc which takes corneal biomechanical properties into account and has been reported to be less affected by corneal properties than other tonometric techniques (Luce, 2005). The mean IOPcc was calculated for each subject at each measurement session from a total of four readings.

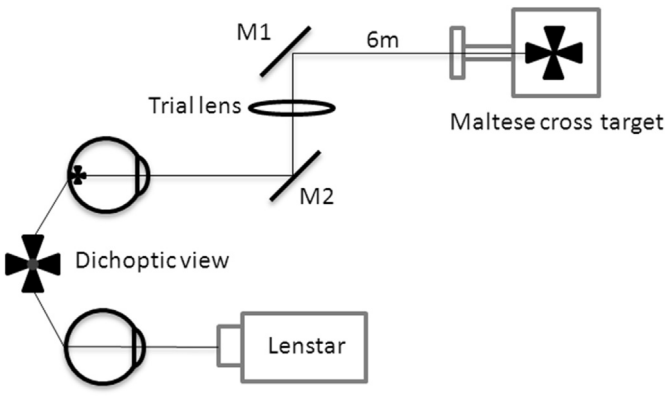

Fig. 1. Schematic diagram of the experimental set-up for ocular biometry measurements using a periscope system (consisting of a pair of mirrors (M1 and M2), and a correcting lens) with the Lenstar and a correcting lens that allows ocular biometry to be measured along the line of sight with relaxed accommodation under binocular dichoptic fixation.

2.3. Data analysis

The retinal and choroidal thickness data obtained from the SOCT Copernicus HR were extracted from the OCT images and calculated with a two-step method. In the first step, all 30 individual scans along the horizontal and vertical scan lines in each series of scans were aligned to provide average horizontal and vertical B-scan images with reduced speckle noise and increased visibility of the retinal layers and the chorioscleral interface (Alonso-Caneiro et al., 2011). In the second step, an experienced masked observer manually segmented the average B-scan images (3 horizontal and 3 vertical images at each measurement session) to derive retinal and choroidal thickness. The observer selected 16 points along three boundaries in each OCT image: the inner boundary of the inner limiting membrane (ILM), the outer boundary of the RPE and the inner border of the chorioscleral interface across the 6 mm length of each average image and the software automatically fitted a smooth function (spline fit) to these points. The observer also manually selected the center of the fovea on each average OCT scan.

Since the transverse resolution of OCT images is influenced by AL (Odell et al., 2011), the transverse scale of the images was adjusted based upon the participant's AL obtained with the Lenstar (Lenstar LS 900; Haag Streit AG) using an approach similar to that described by Wagner-Schuman et al. (2011).

Based on the manual segmentation, the average foveal retinal thickness (distance between the ILM and the RPE at foveal centre) as well as the average subfoveal and parafoveal ChT (distance between the outer boundary of the RPE and the inner boundary of the chorioscleral interface, in nasal, superior, temporal and inferior quadrants, extending to 1.5 mm from the fovea average at 0.5 mm intervals) were calculated at each measurement session. The individual sectors were referred to as the "average subfoveal choroidal thickness" (for the thickness at the center of the fovea), nasal, superior, temporal and inferior parafoveal thickness (for the average ChT from 0.5 mm away from foveal center to 1.5 mm from the foveal center). Fig. 2 illustrates an example of an average OCT image from a representative subject, along with an overview of the retinal and choroidal segmentation and analysis procedure used in the study.

Following data collection, the average of all the biometric parameters (AL, CCT, corneal curvature, ACD, LT, RT, subfoveal ChT and parafoveal ChT) and IOP for each participant at each measurement session were calculated. The Kolmogorov–Smirnov test revealed that data from all variables did not depart significantly from a normally distributed sample ($p > 0.05$). A repeated-measures analysis of variance (ANOVA) with two within-subject factors (time and drug) was carried out for each of the measured variables to determine the significance of changes in each of the ocular parameters after instillation of the pharmaceutical agents. Bonferroni corrected pairwise comparisons were used to examine individual differences for any variables with significant within–subject effects and interactions. Additionally, for the parafoveal choroidal thickness data, two additional within-subject factors (parafoveal region [0.5 mm, 1 mm, 1.5 mm from the fovea center] and quadrant [temporal, nasal, superior and inferior]) were included in the ANOVA.

To provide an assessment of the within-session precision of each of the ocular parameters measured, the within-session SD and coefficient of variation (i.e., the within-session SD divided by the mean, expressed as a percentage) of the repeated measures for each variable collected at each session were calculated as described by Bland and Altman (1999). The intraclass correlation coefficient (ICC) was also calculated for each of the ocular parameters. The ICC is an index of measurement reliability that ranges from 0 to 1, with values of the ICC >0.75 having been suggested to represent excellent measurement reliability; ICC ≥0.4 represents good reliability and ICC< 0.4 to be poor (Portney and Watkins, 2008).

Table 1
Summary of within-session repeatability for each of the ocular variables measured at each measurement session.

	Mean within-session standard deviation	Mean coefficient of variation (%)	ICC
AxL (mm)	0.007	0.03	0.998
CCT (mm)	0.002	0.35	0.998
ACD (mm)	0.011	0.41	0.998
LT (mm)	0.014	0.45	0.996
Subfoveal ChT (mm)	0.002	0.78	0.998
Parafoveal (0.5 mm) ChT (mm)	0.002	0.84	0.998
Parafoveal (1.0 mm) ChT (mm)	0.003	0.98	0.997
Parafoveal ChT (mm)	0.004	1.65	0.992
RT (mm)	0.003	1.45	0.996
Keratometry (D)	0.165	0.38	0.987
IOPcc (mm Hg)	1.158	8.32	0.903

3. Results

3.1. Within-session repeatability

The within-session SD and coefficient of variation generally indicated highly precise measures for each of the ocular measurements. The within-session SD ranged from 0.005 to 0.02 mm for ocular biometrics (AL, CCT, ACD and LT), 0.002–0.012 mm for subfoveal and parafoveal ChT, 0.003 mm for RT, 0.169 D for corneal curvature, and 1.15 mm Hg for IOPcc. ICC analysis suggested "excellent" reliability for all variables (ICC > 0.90 for all variables). Table 1 illustrates the average within-session variability for each of the ocular variables across all measurement sessions.

3.2. Subfoveal choroidal thickness

Repeated measures ANOVA demonstrated a significant effect of drug, and drug by time interaction for the change in subfoveal

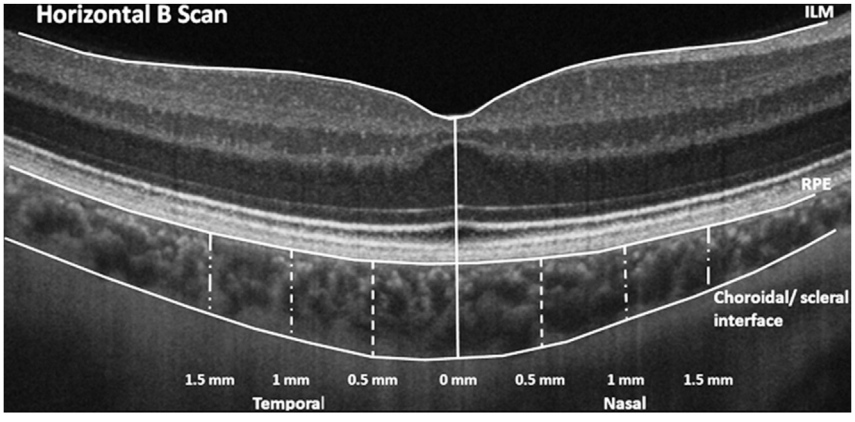

Fig. 2. Example of a representative average OCT image from one subject (derived from the average of 30 individual B-Scans), and the analysis procedure performed on the images captured (i.e. 3 vertical and 3 horizontal average images at each measurement session) for each subject. Manual segmentation defined the inner limiting membrane, the outer border of the RPE and inner border of the chorioscleral interface (white lines) in each image. Retinal thickness was defined as the distance from the inner limiting membrane and the outer border of the RPE. The subfoveal and parafoveal choroidal thickness (white continuous and dotted lines in the average B-scan respectively) were determined as the distance from the RPE to the chorioscleral interface in each of the manually segmented B-scans. White bars represent the choroidal thickness measurements at the fovea and at 0.5 mm intervals up to 1.5 mm.

choroidal thickness from baseline (both $p < 0.0001$). Table 2 presents the subfoveal choroidal thickness results for all subjects. The instillation of 2% homatropine resulted in a small but significant increase in subfoveal ChT (mean change of 7 ± 3 μm, $p < 0.0001$ and 14 ± 2 μm, $p < 0.0001$) after 30 and 60 min respectively (Fig. 3). Administration of 2.5% phenylephrine produced a small decrease in ChT with a mean change of −1 ± 2 μm after 30 min that was not statistically significant ($p = 0.6$) and a mean change of −2 ± 2 μm after 60 min that bordered on statistical significance ($p = 0.053$). No significant changes in the subfoveal ChT from the baseline were found with the placebo at any examination points (mean change 0 ± 1 μm and 0 ± 0 μm for 30 and 60 min respectively; $p > 0.05$). There was also no significant difference between the baseline subfoveal ChT measurements (prior to drug instillation) for any of the three conditions tested on different days (mean baseline ChT were 343 ± 8 μm, 343 ± 8 μm and 344 ± 8 μm for the sessions for 2% homatropine, 2.5% phenylephrine and placebo respectively).

3.3. Parafoveal choroidal thickness

Table 3 shows the mean changes in the parafoveal choroidal thickness for the four (temporal, nasal, superior and inferior) quadrants in 0.5 mm regions located 0.5, 1 and 1.5 mm from the center of the fovea. Repeated measures ANOVA revealed the parafoveal choroid exhibited a similar increase in thickness as that observed in the subfoveal choroid with time across all regions after 2% homatropine administration (drug by time interaction $p < 0.001$). Fig. 4 shows the mean choroidal thickness changes observed in the different parafoveal regions (averaged across all quadrants) at 30 and 60 min post-instillation.

Table 2
Effects of 2% homatropine, 2.5% phenylephrine and placebo on the mean change in ocular parameters at 30 and 60 min from the baseline. Statistically significant ANOVA main effects and interactions ($p < 0.05$) are highlighted in bold. Asterisks indicate significant changes from baseline in the ocular parameter, from the Bonferroni corrected pairwise comparisons ($p < 0.05$).Positive values represent an increase in choroidal thickness, while the negative values correspond to a decrease in choroidal thickness.

	Mean change from baseline (Mean ± SD)			ANOVA *p* value	
	Drug				
	2% homatropine	2.5% phenylephrine	Placebo	Drug	Drug by time
AL(μm)				0.723	**0.001**
30 min	−4 ± 6	2 ± 4	2 ± 7		
60 min	−6 ± 6*	3 ± 5	3 ± 6		
CCT (μm)				0.557	0.380
30 min	0 ± 2	0 ± 3	0 ± 2		
60 min	−1 ± 4	2 ± 5	0 ± 2		
ACD (μm)				0.632	**0.011**
30 min	41 ± 25	14 ± 14	−1 ± 9		
60 min	57 ± 28*	23 ± 20	−2 ± 9		
LT (μm)				0.549	**0.047**
30 min	−20 ± 23	−2 ± 6	4 ± 10		
60 min	−25 ± 27*	−4 ± 8	5 ± 9		
RT (μm)				0.235	0.391
30 min	1 ± 3	1 ± 2	1 ± 4		
60 min	1 ± 3	1 ± 3	0 ± 4		
Subfoveal ChT (μm)				**<0.0001**	**<0.0001**
30 min	7 ± 3*	−1 ± 2	0 ± 1		
60 min	14 ± 2*	−1.5 ± 2	0 ± 0		
Keratometry (D)				0.456	0.251
30 min	0.01 ± 0.21	0.02 ± 0.19	0.01 ± 0.15		
60 min	0.05 ± 0.22	0.03 ± 0.21	0.03 ± 0.18		
IOP (mm Hg)				0.485	0.168
30 min	0 ± 2	0 ± 2	−1 ± 1		
60 min	1 ± 1	1 ± 1	−1 ± 2		

Overall, there was a very small decrease in choroidal thickness 30 and 60 min after administration of 2.5% phenylephrine in all parafoveal regions; however the changes were not statistically significant ($p > 0.05$). Similarly, there was no statistically significant change in parafoveal choroidal thickness after placebo instillation at the 30 and 60 min post-instillation measurements ($p > 0.05$ for all comparisons).

3.4. Retinal thickness

Table 2 shows the mean change in foveal retinal thickness (RT) from baseline for the three experimental conditions. On average, retinal thickness at the fovea did not change in response to either drug (average RT change was <1 μm, $p > 0.05$ for drug) and there were no significant drug by time interactions detected ($p > 0.05$).

3.5. Ocular biometry

Details of the mean ocular biometric changes following use of the three different drugs are illustrated in Table 2. On average, none of the anterior eye biometric measures (AL, CCT, corneal curvature, ACD, and LT) exhibited significant changes after instillation of 2.5% phenylephrine or placebo at both 30 and 60 min ($p > 0.05$ for all comparisons). In contrast, 60 min after administration of 2% homatropine the axial length (AL) was significantly shorter (mean change, −6 ± 6 μm; $p = 0.001$, drug by time interaction). The anterior structures of the eye also demonstrated significant changes after homatropine instillation, with anterior chamber depth (ACD) deepening (mean change 41 ± 25 μm; and 57 ± 28 μm for 30 and 60 min respectively) and the crystalline lens (LT) thinning (mean change −20 ± 23 μm and −25 ± 28 μm). Pairwise comparisons showed these changes were significant for ACD and LT at 60 min only ($p < 0.05$) (Table 2). IOP did not significantly change after the introduction of any of the three pharmacological agents.

4. Discussion

The results of this study demonstrate that the anticholinergic agent homatropine can increase choroidal thickness (7–14 μm) in young healthy adults in both foveal and parafoveal regions, presumably by modifying choroidal autonomic nervous system activity. To our knowledge, this is the first study to examine the influence of homatropine upon choroidal thickness in human subjects. Although the changes found were of small magnitude, they highlight the importance of muscarinic mechanisms in the regulation of choroidal thickness in human subjects.

The exact mechanism driving these changes is unclear; however, alterations in the tone of choroidal non-vascular smooth muscle or changes in choroidal blood flow have been suggested as two likely causes of choroidal thickening after administration of antimuscarinic drugs in animals (Nickla and Wallman, 2010). Evidence of contraction of choroidal non-vascular smooth muscle in response to increased parasympathetic stimulation (Meriney and Pilar, 1987), and the recent finding of choroidal thickening following double parasympathectomy in chicks (Nickla and Schrödl, 2012), suggests an involvement of parasympathetic innervation and non-vascular smooth muscle in the choroidal thickening response. These findings support a potential role of non-vascular smooth muscle in the choroidal thickness changes that we have observed with homatropine in humans, whose choroidal non-vascular smooth muscle also has parasympathetic innervation (Nickla and Wallman, 2010).

In contrast, it is generally accepted that acetylcholine (ACh) released from parasympathetic cholinergic nerves is a powerful

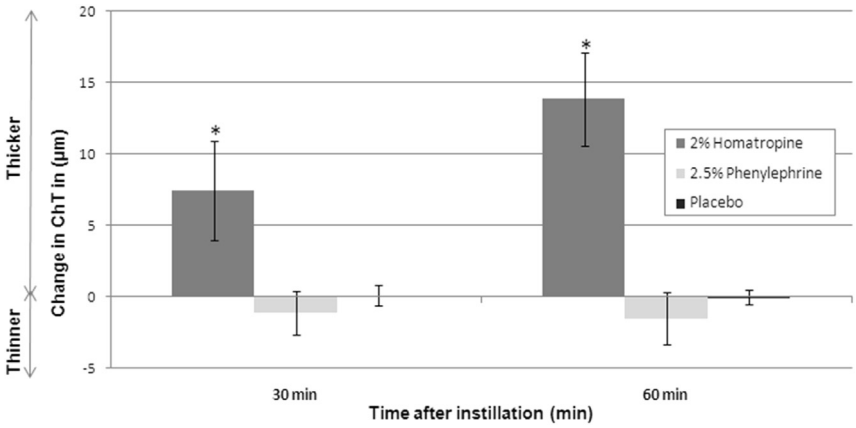

Fig. 3. Mean change from baseline in subfoveal ChT at 30 and 60 min after drug instillation. Pairwise comparison revealed a significant increase in choroidal thickness at 30 and 60 min after 2% homatropine administration (asterisk shows $p < 0.0001$) when compared to 2.5% phenylephrine and placebo (both $p > 0.05$). Error bars represent ± SD.

Table 3
Mean change in parafoveal choroidal thickness from the baseline after 30 and 60 min of exposure to three different drugs for four choroidal quadrants within three different regions. Asterisks indicate significant changes from baseline in the ocular parameter, from the Bonferroni corrected pairwise comparisons ($p < 0.05$). Positive values represent an increase in choroidal thickness, while the negative values correspond to a decrease in choroidal thickness.

Quadrant	Region (mm)	Time	Change from baseline (µm) mean ± SD		
			Drug		
			2% Homatropine	2.5% Phenylephrine	Placebo
Temporal	0.5	30	7 ± 4*	0 ± 1	0 ± 1
		60	14 ± 4*	−1 ± 2	0 ± 1
	1.0	30	6 ± 3*	0 ± 2	0 ± 2
		60	14 ± 3*	0 ± 2	0 ± 3
	1.5	30	7 ± 2*	−1 ± 1	0 ± 0
		60	13 ± 3*	−1 ± 3	−1 ± 4
Nasal	0.5	30	7 ± 4*	−1 ± 1	0 ± 2
		60	11 ± 4*	−1 ± 2	0 ± 1
	1.0	30	7 ± 6*	0 ± 2	−1 ± 2
		60	13 ± *6	−1 ± 2	−1 ± 2
	1.5	30	7 ± 3*	0 ± 2	0 ± 1
		60	13 ± 5*	1 ± 3	−1 ± 2
Superior	0.5	30	6 ± 3*	−1 ± 1	0 ± 1
		60	13 ± 4*	−1 ± 1	0 ± 1
	1.0	30	6 ± 3*	0 ± 2	−1 ± 2
		60	13 ± 2*	−1 ± 1.	−1 ± 2
	1.5	30	4 ± 3*	0 ± 1	0 ± 1
		60	10 ± 3*	−1 ± 1	0 ± 2
Inferior	0.5	30	7 ± 4*	0 ± 1	0 ± 1
		60	13 ± 4*	−1 ± 1	0 ± 1
	1.0	30	5 ± 4*	−1 ± 2	1 ± 2
		60	11 ± 3*	−1 ± 2	0 ± 1
	1.5	30	5 ± 5*	−1 ± 1	1 ± 2
		60	11 ± 5*	−1 ± 1	−1 ± 2

dilator of vascular beds that is primarily dependent upon the release of endothelial nitric oxide (Toda and Nakanishi-Toda, 2007), which would predict a vasoconstriction (and hence a possible thinning of the choroid) in response to anticholinergic agents. However, there is evidence from animal studies that anticholinergic drugs such as atropine cause a vasodilation response in ocular blood vessels, potentially through their action on pre-junctional muscarinic receptors, thus increasing the release of neural nitric oxide (Ayajiki et al., 2000). Therefore, it is possible that the mechanism involved in choroidal thickening observed in our study could also involve the presynaptic inhibition of muscarinic receptors by homatropine, resulting in increased neural NO release, and subsequent relaxation of smooth muscle.

In animal studies, choroidal thickness and ocular growth changes have been shown to be influenced by non-selective, partially selective or highly selective muscarinic antagonists (i.e., atropine, pirenzepine, himbaicine, MT3, MT7) implicating muscarinic involvement in eye growth and myopia control (Arumugam and McBrien, 2012; Luft et al., 2003; Diether et al., 2007). Results from human clinical trials also indirectly support this hypothesis, with outcomes demonstrating that daily administration of the non-selective muscarinic antagonist 1% atropine, reduced the progression of myopia by about 60% during the second year of treatment, and concentrations as low as 0.01% atropine still reduced eye growth by 50% (Chua et al., 2006; Chia et al., 2012). In our study, the change in ChT of the two myopic subjects in response to 2% homatropine (14.1µm ± 3.1 at 60 min) was not substantially different to the response of the remaining 12 emmetropic subjects (13.7µm ± 3.5 at 60 min). Our study outcomes add to the evidence that the muscarinic signalling blockage can also influence choroidal thickness in the human eye, at least in the short term. Further research is required to understand if there is a relationship between these short term choroidal thickness changes in response to anticholinergic agents and longer term changes in eye growth and refractive error development.

The topical administration of 2.5% phenylephrine caused only a small decrease in choroidal thickness that was not statistically significant. Increased activity of sympathetic adrenergic nerves may be predicted to result in choroidal thinning predominantly due to the contraction of vascular muscle cells that contain alpha-adrenergic receptors. However, the exact role of non-vascular smooth muscle cells in this process remains unknown. Previous studies of the effect of phenylephrine on the human eye have produced conflicting results, with some authors observing a decrease in choroidal thickness in both the treated and fellow eyes after topical phenylephrine administration (Kara et al., 2014), whereas others have reported no change in choroidal thickness (Bajenova et al., 2012). Similarly, reports on changes in choroidal blood flow as a result of stimulation of the sympathetic system are also equivocal with both reductions in ocular blood flow (Lanigan et al., 1988; Takayama et al., 2009) and no change in posterior eye circulation in healthy human subjects reported (Takayama et al., 2004; Robinson et al., 1985; Schmetterer et al., 1996), In our study, the lack of statistically significant changes in choroidal thickness may suggest that phenylephrine did not reach the posterior pole at concentrations sufficient to induce a direct vasomotor effect. There are reports (Maurice and Mishima, 1984; Del Amo and Urtti, 2008) suggesting that topical administration of the various

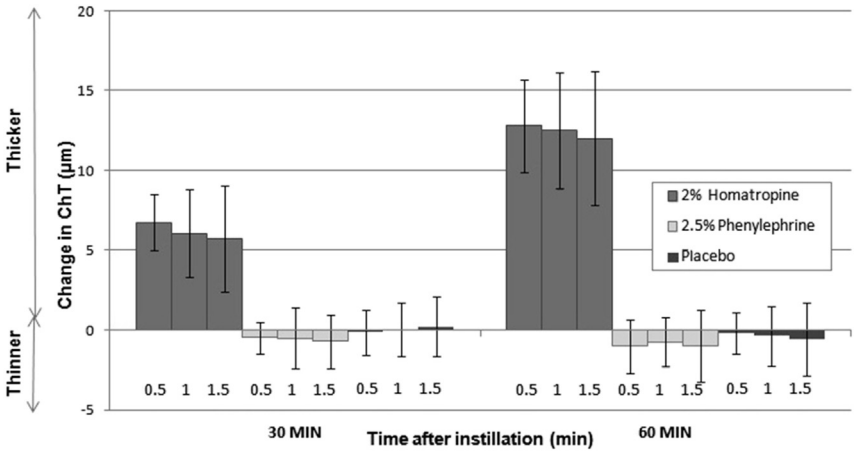

Fig. 4. Mean change from baseline in parafoveal ChT 30 and 60 min after drug instillation for average of 0°, 90°, 180° and 270° measurement meridians. Error bars represent ± SD. The values under each of the bars on the *x* axis represent the distance in mm from the foveal center where each of the parafoveal choroidal thickness measurements were taken.

mydriatic agents may not reach concentrations necessary to exert an effect at the posterior pole. As the findings from human experiments with phenylephrine have provided contradictory results, further studies exploring the potential vasoconstrictive effects of adrenergic stimulants on choroidal blood vessels and choroidal thickness changes in human eyes may be warranted.

To our knowledge only one study (Mwanza et al., 2013) has previously examined the effect of a muscarinic antagonist on parafoveal choroidal thickness and they reported no significant difference in choroidal thickness after administration of 1% tropicamide in healthy individuals and glaucoma patients. Contrary to these findings by Mwanza et al. (2013), we did find that topical administration of homatropine produced increases in both subfoveal and parafoveal choroidal thickness. These differences in choroidal responses to antimuscarinic agents may represent a difference in the posterior segment bioavailability of these two drugs (Smith, 1976). The mechanism by which topically applied drugs can reach the intraocular environment is through both a corneal and a conjunctival/scleral penetration route. Penetration across the cornea is proposed as the primary pathway by which drugs reach the anterior segment after topical administration, whereas the conjunctiva/scleral route is more important to allow access into more posterior structures of the uveal tract (Hughes et al., 2005). The relative contribution of this route is more important in case of drugs that poorly penetrate across the cornea (Heier et al., 2009). Since tropicamide shows a low affinity and quick corneal penetration (Smith, 1976), it is possible that the dose of the drug reaching the posterior eye may be too low to induce choroidal thickness changes.

Significant changes in choroidal thickness after treatment with 2% homatropine were also accompanied by changes in axial length observed 60 min after drug administration. The magnitude of these changes was relatively small but statistically significant, with an average of 6 μm of decrease in axial length after the muscarinic antagonist administration, with the direction of change (shortening) being consistent with previous studies in both avian species (Nickla and Schrödl, 2012) and mammals (Arumugam and McBrien, 2012). Since the sub-foveal choroid increased in thickness by 14 μm and the axial length decreased by 6 μm 60 min after homatropine instillation, the unaccounted 8 μm may be the result of the biomechanical forces on the globe associated with ciliary muscle relaxation and changes in the iris that also led to significant deepening of the anterior chamber and lens thinning. Previous animal (Nickla and Schrödl, 2012; Lin et al., 1996; Schmid and Wildsoet, 1996) and human (Drexler et al., 1997; Cheung et al., 2009) studies have also reported a significant variation in the anterior chamber depth (ACD) and lens thickness (LT) measurements after the administration of muscarinic antagonists or surgical modification of parasympathetic input to the eye, with trends similar to the results obtained in our study. Pharmacologic blockade of cholinergic transmission resulted in significant enlargement of ACD and thinning of the lens. In our data, the magnitude of LT thinning was smaller than ACD deepening, suggesting a possible posterior displacement of the lens.

Natural variations in choroidal thickness over the course of the day, eye diseases or topical and systemic medications should be considered as potential confounders in any studies reporting choroidal thickness. Previous studies have found that the choroid and axial length undergo significant diurnal fluctuations (Stone et al., 2004; Brown et al., 2009; Chakraborty et al., 2011). The choroid is typically thickest in the evening and thinnest around noon, while AL changes are antiphase to the ChT variations. To ensure consistency and minimize any influence from potential diurnal variation, we tested all conditions at approximately the same time of the day. Although our study included only health young adult subjects, our findings highlight the potential importance of muscarinic mechanisms in the regulation of choroidal thickness in humans. Future research, particularly in older subjects with disease (who are more likely to be using medications) should consider the potential for topical and/or systemic drugs that can influence the muscarinic system, to be potential confounders in studies of choroidal thickness.

5. Conclusions

Instillation of the anticholinergic agent 2% homatropine resulted in an increase in subfoveal and parafoveal choroidal thickness in young healthy adult subjects. Therefore the study results provide important insight into the potential role of muscarinic mechanisms in transient choroidal thickening in the human eye.

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Conflict of interest

The authors have no financial or conflicting interest to disclose.

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